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ORIGINAL ARTICLE

Thiopurine methyltransferase polymorphisms in a Brazilian population

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ABSTRACT
Thiopurine methyltransferase (TPMT) catalyses the S-methylation of thiopurine drugs. Low-activity phenotypes are correlated with several mutations in the TPMT gene. Polymorphisms of TPMT have been reported for Caucasians, African-Americans and Asians. Since ethnic differences have been demonstrated worldwide, it remains to be elucidated in Brazil. The Brazilian population is the result of five centuries of interethnic crosses between peoples from almost all continents as well as autochthonous Amerindians, all forming the fifth largest and one of the most heterogeneous populations in the world. The frequency of six allelic variants of the TPMT gene, *2 (G238C) (2.2%), *3A (G460A and A719G) (1.5%), *3B (G460A) (0.2%), *3C (A719G) (1.0%), *5 (0%) and *6 (0%) were determined in Brazilian subjects using polymerase chain reaction (PCR)-RFLP and allele-specific PCR-based assays. This study provides the first analysis of TPMT mutant allele frequency in a sample of the Brazilian population.
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INTRODUCTION

The anticancer prodrugs 6-mercaptopurine (6-MP) and azathioprine (AZA) are widely used to treat several diseases such as childhood acute lymphoblastic leukemia (ALL), autoimmune hepatitis, myasthenia gravis and rheumatoid arthritis.[1–5] However, serious toxicity following 6-MP administration has been associated with genetic polymorphisms of the thiopurine *S*-methyltransferase (*TPMT*) gene. TPMT is a cytoplasmic enzyme (*S*-adenosyl L-methionine : thiopurine *S*-methyltransferase; EC 2.1.1.67) that preferentially catalyzes *S*-methylation of AZA, 6-MP and thioguanine, inactivating 6-MP and 6-thioguanine by *S*-methylation at position C-6 of the purine ring,[6] but there is a large interindividual variability in the rate of S-methylation of these thiopurine drugs. The *TPMT* gene is localized to chromosome 6p22.3 and consisting of either 10 or nine exons.[7,8] TPMT is inherited as an autosomal codominant trait [9,10] and exhibits a genetic polymorphism with 1/300 individuals having complete deficiency and about 10% of Caucasians having intermediate activity because of heterozygosity.[11–13] The molecular basis for low TPMT activity has been elucidated with the identification of the wild-type (WT) allele TPMT*1 and three nonsynonymous single-nucleotide polymorphisms accounting for the majority of mutant alleles.[14–17] Patients with low or intermediate enzyme activity are at risk to develop severe hematopoietic toxicity after receiving standard doses of thiopurine medications.[18] TPMT phenotype has been assigned on the basis of its activity in erythrocytes as previously described,[19] but these measurements are laborious and vary with red blood cell age.[20] Furthermore, bimodal or trimodal activity patterns have been shown in several populations.[16,21–34] Since ethnic

differences have been demonstrated worldwide, it remains to be elucidated in Brazil, the fifth largest populated country in the world. The present-day Brazilian populations reflects the result of five centuries of interethnic crosses between peoples from almost all continents (Europeans, African, Asians) as well as autochthonous Amerindians, all forming one of the most heterogeneous populations in the world.[35,36] In the only reported data about the distribution of TPMT activity in the Brazilian population, the enzyme activity in erythrocytes was measured in 134 randomly selected black, white, mixed-race and Japanese subjects living in Brazil.[37] It has been estimated that there will be approximately 2000 new cases of childhood ALL in Brazil (with a rate of cure of around 80%).[38] As the administration of purine analogues are the part of the treatment design for ALL, analysis of the *TPMT* gene to prevent the severe toxicity associated with these compounds is of great importance to optimize their use. Therefore, in the present study we determined the *TPMT* genotype in 202 randomly selected, unrelated, Brazilian subjects and in two patients with ALL who had severe drug side effects requiring 6-MP dose reduction.

RESULTS

TPMT genotypes were determined for all subjects with PCR methods used to detect the most prevalent mutant alleles (TPMT*2, TPMT*3A, TPMT*3B, TPMT*3C, TPMT*5 and TPMT*6). Mutant TPMT alleles were found in 9.3% (20 in 204) of Brazilian individuals. TPMT*2 was present in seven subjects, five being heterozygous and two homozygous, with an allele frequency of 2.2% (Table 1). The second most common mutant allelotype was TPMT*3A, with a frequency of 1.5%. Table 1 summarizes all mutant alleles found in this Brazilian population. Table 2 shows the TPMT genotypes of all 20 mutant TPMT subjects and their ethnicity. Both ALL patients were heterozygous for the mutant alleles, one for TPMT*2 and other for TPMT*3C. Figure 1a shows the DNA restriction enzyme digest with Acc I (A719G-TPMT*3C) from one affected patient with ALL and normal controls. Figure 1b shows digestion with *Mwo*I (G460A-TPMT*3B) with one heterozygous patient (last two lanes). Figure 1c shows the PCR amplification product with primers (WT-specific reaction) and MP (mutant-specific reaction) (G238C-TPMT*2) of homozygous subjects and one TPMT*2 heterozygous patient with ALL that presented severe toxicity towards 6-MP.

Table 1 Allelic frequencies of TPMT variants in a sample of 204 Brazilian subjects

Allele	*N*	*Percentage of alleles*
No. of alleles	408	—
*2	9	2.2
*3A	6	1.5
*3B	1	0.2
*3C	4	1.0
*5	0	0
*6	0	0

Table 2 Population ethnicity and TPMT alleles in a Brazilian population

	*TPMT*2*	*TPMT*3A*	*TPMT*3B*	*TPMT*3C*
White	3	1	1	4
Non-white	4	2	3	3

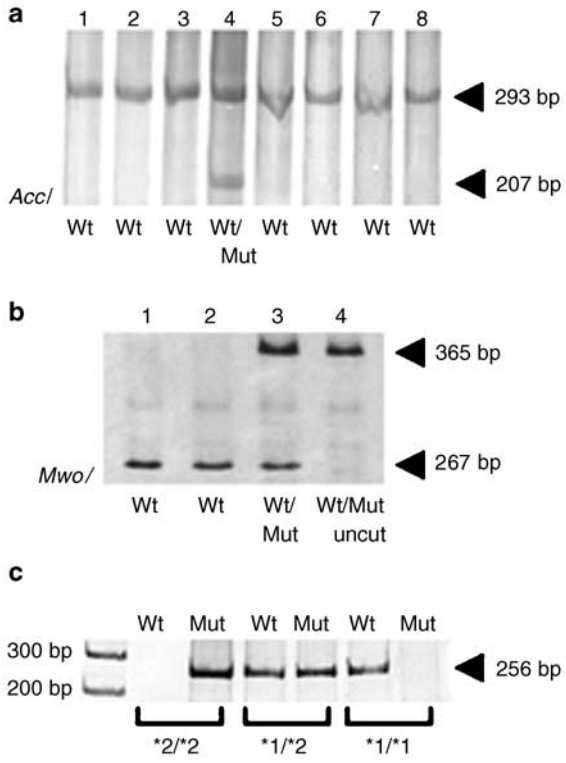

Figure 1 Electrophoretic pattern for TPMT alleles (WT, wild type; Mut, mutant). TPMT alleles were analyzed by PCR-based methods. (a) detection of the TPMT *3C allele using the *Acc*I restriction enzyme digestion, which yields fragments of 207 and 87 bp (not shown), showing a heterozygous pattern (lane 4). (b) *Mwo*I digestion to detect the TPMT *3B allele yields fragments of 267 and 98 bp (data not shown). Lane 3 shows a heterozygous genotype with partial digestion and lane 4 is this indigested fragment. (c) Electrophoretic pattern for TPMT alleles were analyzed by PCR with WT primers (WT = P2W+P2C) and mutated primers (Mut = P2M+P2C) for the detection of the TPMT *2 allele as described (Yates *et al*, 1997). Lanes 1 and 2 show a homozygous pattern with amplification only with Mut primers. Lanes 3 and 4 shows a heterozygous pattern with amplification with both primers (patient with ALL who had been treated with 6-MP and presented severe toxicity) and lanes 5 and 6 shows a homozygous WT with amplification with only WT primers.

DISCUSSION

One of the best examples of the application of pharmacogenetics to clinical practice is the genetic polymorphism of

the thiopurine *S*-methyl transferase (TPMT). It has been demonstrated a high degree of concordance between TPMT genotype and phenotype in Caucasians[11,27] and the presence of mutant alleles are predictive of the phenotype, such as heterozygous patients have intermediate activity and homozygous patients have low activity, although a variability can be seen between these groups.[2,3,13,39] It has been known that Africans have around 20% less TPMT activity than Caucasians for the same allelic distribution.[10] In addition, it has been shown a trimodal allelic distribution in Caucasians, although Asians and Koreans have a unimodal distribution.[6,10,24,29,33] However, it must be noted that when determining the presence of polymorphisms in a population, ethnic and racial mixture must be taken into consideration. Thus, it is of the greatest importance to establish the genetic basis for the TPMT polymorphism in various populations, including Brazilians. Indeed, it has been shown that Brazilians comprise one of the most heterogeneous people. In 1500, Brazil was inhabited by 2.4 million Amerindians and, since then, ~4 million African slaves and ~6 million Europeans immigrated to the country.[36] Our data show that all four most frequent mutant alleles are present in the Brazilian population. The WT allele TPMT*1 was the most prevalent, in agreement with several previous reports.[4,6,22–25,29,33] Interestingly, in our sample the TPMT*2 (G238C) accounts for a significant number of mutant alleles. This mutation has been found only in Caucasians and is thought to represent a more recent allele.[23,24] Therefore, based on our data, it remains to be established whether the TPMT*2 is present only in Caucasians or could be found in indigenous people living in the area of what is now Brazil. The TPMT*3A allele (G460A plus A719G) is most prevalent in Caucasians[11,13,22,23,25] and would be expected to be found in our population, as present-day Brazilians carry the genetic imprint of European ancestry in the paternal line as reflected by Y-chromosome markers.[36] The TPMT*3C allele (A719G), with a frequency of 1.0%, has been previously shown in several other ethnic groups including the Saamis, Kenyans, Ghanaians, African-American and Asians.[22–24,29,30] Finally, the very uncommon allele TPMT*3B (G460A) was found in only one subject, thus indicating this allele to be a rare one.[11,16,23] To date, of the two strategies used to identify TPMT-deficient and heterozygous patients, TPMT genotype can easily be performed. Given that all major TPMT mutant alleles are present in the Brazilian population, it would be a good clinical practice to genotype all of them as previously suggested.[40] To avoid 6-MP concentration-dependent side effects, genotyping tests before the initiation of therapy could identify patients with an unacceptable mortality and/or morbidity risks. Indeed, we were able to identify, albeit retrospectively, that both patients with ALL who had severe drug side effects necessitating dose reductions, were heterozygous for the mutant alleles TPMT*2 or TPMT*3C. The percentage of weeks during which 6-MP was decreased to prevent toxicity was 23% in the TPMT*3C heterozygous patient and 26% in the TPMT*2 heterozygous patient. Furthermore, both patients could be treated without toxicity after a 6-MP dose adjustment to one-third of the initial maintenance dose (75 mg/m^2 per day). This allowed administration of full protocol doses of other chemotherapy. The demonstration that TPMT heterozygote developed toxicity is in agreement with the report of *Relling* et al,[2] which described a cumulative incidence of 6-MP dose reduction of 35% among heterozygous patients as compared with 7% among WT patients. In contrast, McLeod *et al*[26] did not find more toxicity in TPMT heterozygote (9.5%) as compared with WT patients (11%). These contradictory findings could be because of the other components of ALL therapy. We used in addition to 6-MP and methotrexate, intercalated cycles of cytarabine plus L-asparaginase or vincristine plus prednisone.

In summary, in this report we determined, for the first time, the frequency of all major TPMT mutant alleles present in a Brazilian sample, a highly heterogeneous population, reinforcing the importance of taking ethnicity into account when studying polymorphisms.

MATERIAL AND METHODS

We investigated 202 successive, unrelated Brazilian subjects (age 18 years and over) who attended the Hospital das Clinicas da UFMG. None of these individuals had a diagnosis of leukemia. In addition, in order to substantiate our data, verifying the concordance between phenotype and genotype, DNA was extracted from the bone marrow of two white patients with childhood common ALL who presented toxicity, necessitating a dose reduction to one-third of the initial maintenance dose of 6-MP. TPMT red blood cell activity from these two patients was unavailable. An informed consent was obtained from all participants and the study was approved by the University Ethics Committee. Ethnicity was determined by self-report as determined by Instituto Brasileiro de Geografia e Estatística (IBGE).[41] However, the hazards of judging Brazilians by color, race and geographical origin have been recently demonstrated.[42]

Genomic DNA was isolated from peripheral blood sample using the GenomicPrep Blood DNA Isolation Kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). For *TPMT* gene analysis, oligonucleotides (Invitrogen Brasil, São Paulo, BR, Brazil) and PCR amplifications were performed as previously described.[11] In addition, further sets of primers were also used.[16] PCR was performed in a final volume of 50 µl containing 100 ng of template DNA, 200 µmol/l dNTPs, 10 pmol/l of each primer, 1.25 U *Taq* polymerase in buffer (10 mmol/l Tris HCl [pH 9], 50 mmol/l KCl, 0.1% Triton X-100, 1.5 mmol/l $MgCl_2$). Amplification was performed in a Mastercycler gradient (Eppendorf, *Germany). Coamplification of β-globin gene served as an internal control for specimen integrity. PCR products were purified with GFX PCR and Gel Band Purification kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) prior to digestion with *Mwo*I (TPMT*3B, G460A) and *Acc*I (TPMT*3C, A719G) restriction enzymes as described by the manufacturer (New England Biolabs, Beverley, MA, USA). Digested samples were electrophoresed in an 8% acrylamide gel and silver stained. Each PCR product was sequenced in an ABI

Prism® 310 Genetic Analyser (Applied Biosystems, Foster City, USA).

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DUALITY OF INTEREST

None declared.

ABBREVIATIONS

ALL acute lymphoblastic leukemia
6-MP 6-mercatopurine
*TPMT **S**-adenosyl L-methionine: thiopurine **S**-methyltransferase*

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